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Conjunctival instillation of scrapie in mice can produce disease

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ABSTRACT

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Mice were infected with one of two strains of scrapie by instilling brain homogenate into the conjunctiva to establish whether infection could be initiated. Of the 55 mice infected with ME7 scrapie, 23 developed clinical disease 323 ± 8 (mean ± standard error) days later. Three out of 12 mice infected with 79A scrapie developed disease after 232 ± 35 days. The ME7 incubation period is similar to that for the oral route of infection. We feel that these results emphasize the need for adequate eye protection when handling tissues infected with spongiform encephalopathies.

INTRODUCTION

Knowledge of the pathophysiology of the transmissible spongiform encephalopathies comes largely from experimental work on scrapie, which has provided guidelines for handling tissues infected with bovine spongiform encephalopathy and Creutzfeldt-Jakob disease (CJD) (Rappaport, 1987). The major route of infection following experimental intravitreal infection of mice with scrapie is via the visual pathways (Fraser and Dickinson, 1985), although a peripheral spread of infection is also initiated (Scott and Fraser, 1989), presumably by inoculum leaking from the injected eye. Removal of the infected eye up to 14 days after intravitreal infection prevents infection reaching the CNS directly via the optic nerve, but mice still develop scrapie after a prolonged incubation period (Scott and Fraser, 1989). We infected mice with scrapie brain homogenates by instilling into the conjunctiva, to mimic the effect of leakage from an intraocular injection, in an attempt to isolate the peripheral component of the pathogenesis. Other peripheral routes,

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such as intraperitoneal infection are known to involve replication in the spleen and lymphoreticular system which then spreads to the CNS, probably via the autonomic pathways (Kimberlin and Walker, 1982).

We also attempted to transfer infection between mouse corneas, as corneal transplants from CJD-infected donors are a known source of iatrogenic transmission (Duffy et al, 1974), and it has been suggested that CJD may have been transferred by the use of a tonometer (Davanipour et al, 1984) which measures ocular pressure.

MATERIALS AND METHODS

The incubation periods and neuropathological changes were studied in mice infected with two strains of scrapie, ME7 or 79A. Inoculum was prepared as a standard 10% saline homogenate of mouse brain terminally-affected by scrapie and estimated to contain between 10^7 and 10^8 intracerebral ID_{50} infectious units per gram. Two mouse strains (SM/RrChBtDk and VL/Dk), which have the same *Sinc* genotype, were infected by dropping 1 μl of the inoculum behind a slightly everted eye under anaesthesia. The *Sinc* gene exerts major control over incubation period length (Bruce and Fraser, 1991). Care was taken to avoid contact between the needle tip, which had been blunted as a precaution, and the ocular tissues. Twenty-eight of the infected mice were enucleated (as a control for intravitreal infection) between 6 hours and 35 days post-infection. Mice were killed when clinical disease became apparent. The intensity of the vacuolar changes was scored in 12 standard brain areas in H&E paraffin sections of formol-fixed brain. The graph of these scores gives the scrapie 'lesion profile' which is used to distinguish the lesion patterns which are characteristic for each strain of scrapie (Bruce and Fraser, 1991).

The possibility that infection could be transferred from the corneal epithelium was tested by swabbing the eyes of 21 C57BL/FaBtDk mice every 5 days or 10 days, with swabs taken from the corneas of mice which were in the final weeks of infection following intracerebral infection with 79A scrapie. The swabbing was continued until the donors became terminal.

RESULTS

In Table 1, the incubation periods of the conjunctivally infected mice are compared with various others resulting from the same dose of infection (between 10^4 and 10^5 intracerebral ID_{50} infectious units) given by other routes (from previous experiments). Of the 55 mice infected with ME7 scrapie, 23 developed clinical disease with an incubation period of 323 ± 8 days. This incubation period is remarkably similar to that for oral infection although 77% of orally-infected mice developed disease compared with 42% with con-

TABLE 1

Comparison of mean incul
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of scrapie. Group numbers
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	ME7 sc
Conjunctival instillation	323 ± 8
Oral	324 ± 4
Intraocular	237 ± 3
Intraocular with enucleation[1]	306 ± 1
Intraperitoneal	303 ± 5
Intracerebral	166 ± 1

[1]Mice were enucleated betwe
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DISCUSSION

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TABLE 1

Comparison of mean incubation periods (days ±SE) following infection of mice of the same *Sinc* genotype with between 10^4 and 10^5 intracerebral ID_{50} infectious units of ME7 or 79A strains of scrapie. Group numbers are shown in brackets: in groups in which not all mice developed clinical disease, the number of positive cases per group is given. These data were collated from more than one experiment

	ME7 scrapie	79A scrapie
Conjunctival instillation	323±8 (23/55)	232±35 (3/12)
Oral	324±4 (23/30)	–
Intraocular	237±3 (n=16)	205±6 (6)
Intraocular with enucleation[1]	306±10 (n=20)	–
Intraperitoneal	303±5 (n=11)	201±2 (9)
Intracerebral	166±1 (n=10)	155±8 (6)

[1]Mice were enucleated between 6 hours and 35 days post-infection; data from Scott and Fraser, 1989.

junctival infection. Enucleation had no effect on the incubation period. Figure 1 shows that the lesion profile from the conjunctivally infected mice is considerably lower than with intraocular or oral routes of infection, especially in the superior colliculus (area 3) and the scoring areas in the prosencephalon (areas 6–9).

Three out of the 12 mice infected with 79A scrapie developed disease after 232±35 days; we have no data on oral infection with this strain of scrapie. Attempts to transmit 79A scrapie from intracerebrally infected to uninfected mice by corneal swabbing were unsuccessful.

DISCUSSION

These studies indicate a close similarity between conjunctival and oral routes of infection. Scrapie incubation periods are strictly dose-dependent, and the efficiency of infection of a given dose varies with route. The survival of a proportion of mice infected by oral and conjunctival routes means that the dose of infection *for these routes* must have been between one and two ID_{50} infectious units. In titrations of ME7 scrapie, groups with survivors occur around 250 days in intracerebrally infected mice, and 330 days in intraperitoneally infected mice.

The change in lesion intensity (Fig. 1) also indicates an alternative pathogenesis. A similar reduction in the severity of lesions is found between intracerebral and intraperitoneal routes, thought to be due to the differences in the accessibility to vital CNS regions of infection following an intraperitoneal infection.

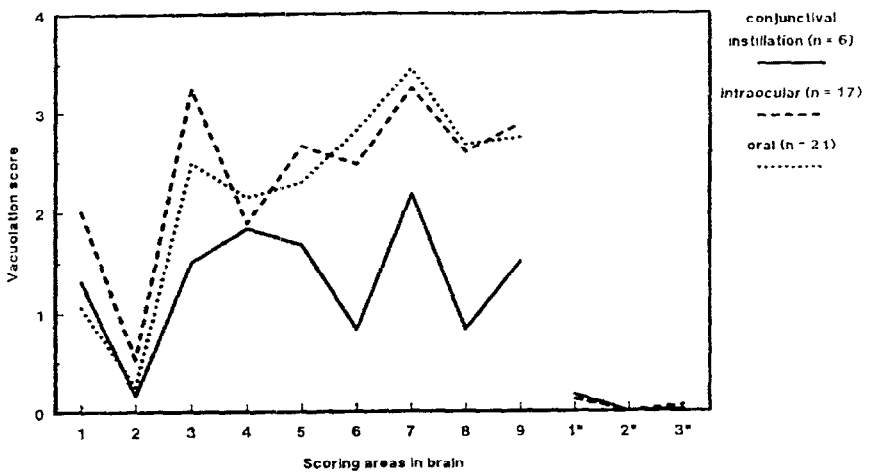

Fig. 1. Comparison of the pattern and severity of vacuolar degeneration in 9 grey matter and 3 white matter areas of brain in mice of the same *Sinc* genotype infected by three different routes.

Although enucleation following intraocular infection indicates that a peripheral infection is initiated when mice are infected by the intraocular route (Scott and Fraser, 1989), the incubation periods for conjunctival infection were unexpectedly precise. The narrow incubation period range with ME7 (the mean of 323 ± 8 ranged from 319 to 337 days) suggests a simple and consistent infectious pathway. In the spongiform encephalopathies in general, peripheral infection usually involves replication in the spleen and lymphoreticular system, and primary replication occurs in lymphoid tissue either draining or close to the site of infection (Hadlow et al, 1987; Kimberlin et al, 1989). Assay of mouse lachrymal gland following intraocular infection indicates that persistant infection is found in this tissue (Scott, unpublished data). Following conjunctival instillation, infection spreading from the lachrymal gland could follow the lymphoid drainage of the conjunctiva (Forrester, 1987) or spread through the lachrymal sac to the back of the throat, thus providing a persistent source of oral infection.

Compared to experimental routes such as intracerebral and intraocular, conjunctival instillation is not an efficient route of infection, as almost 60% of the mice survived. However, apart from oral infection, this is the only non-invasive route of infection for these diseases. We feel that these results demonstrate the necessity for adequate eye protection when handling tissues infected with spongiform encephalopathies.

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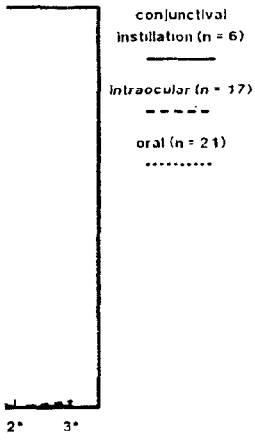

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